

⑫

**EUROPEAN PATENT APPLICATION**

⑰ Application number: **84300805.3**

⑮ Int. Cl.<sup>3</sup>: **A 61 F 1/00, C 08 J 7/04**

⑱ Date of filing: **08.02.84**

⑳ Priority: **27.04.83 US 488911**

㉑ Applicant: **Becton, Dickinson and Company, Mack Centre Drive, Paramus New Jersey 07652 (US)**

㉓ Date of publication of application: **07.11.84**  
**Bulletin 84/45**

㉒ Inventor: **Williams, Joel L., 1306 Walnut Street, Cary North Carolina (US)**  
Inventor: **Dunn, Terry S., 1300 Canterbury Road, Raleigh North Carolina (US)**  
Inventor: **O'Connell, James P., 1909 White Plains Road, Chapel Hill North Carolina (US)**  
Inventor: **Montgomery, David, 801 Brookgreen Drive, Cary North Carolina (US)**

㉔ Designated Contracting States: **BE DE FR GB IT NL SE**

㉕ Representative: **Ruffles, Graham Keith et al, MARKS & CLERK 57-60 Lincoln's Inn Fields, London WC2A 3LS (GB)**

㉖ **Heparinization of plasma treated substrates.**

㉗ Improved antithrombogenicity results when heparin is attached to a polymeric surface activated by treatment with a plasma. The surface thus activated is rendered receptive to the binding of a surface active agent. The surface active agent in turn reacts with heparin to provide a polymeric surface of exceptional antithrombogenicity when contacted with blood for an extended period of time.

**EP 0 124 200 A2**

M&amp;C FOLIO: 799P47184

WANGDOC: 0236C

## HEPARINIZATION OF PLASMA TREATED SUBSTRATES

The present invention relates generally to methods of reducing thrombogenicity associated with polymer resin articles. More particularly, the present invention relates to a method for producing a heparin coated polymeric article wherein the polymeric article is treated with a plasma prior to attachment of the heparin.

US Patent No 3,846,353, to Grotta indicates that polymers, both natural and synthetic and particularly certain synthetic plastics have come to the fore as preferred materials for prosthetic devices. Their major drawback, however, is their thrombogenicity. Even though plastics are used in various apparatus such as heart-lung machines, kidney machines, and artificial heart valves and patches, the tendency of these materials to cause coagulation necessitates the use of anticoagulants such as heparin. Even such plastics as Teflon (trade mark for polytetrafluorethylene) and the silicone rubbers which are more compatible with blood than most plastics, still show thrombogenic characteristics.

The first real advance in the preparation of nonthrombogenic materials was described by Dr Vincent Gott. The method used by Dr Gott comprised treating a graphited surface first with Zephiran (trade mark for benzalkonium chloride) and then with heparin. Materials treated in this way were nonthrombogenic in vivo for long periods of time.

The major disadvantage, however, with these materials, was that the method could only be practiced on rigid plastics. Thus a need still exists for a suitable flexible nonthrombogenic plastics material, as well as a method for producing it.

Various methods for introducing antithrombogenicity involve chemically bonding a quaternary ammonium salt to a polymeric surface. Illustrative procedures are given in US Patent No 3,634,123 to Eriksson wherein an article having a plastics surface is heated to near or above its softening point in an aqueous solution of a cationic surface active agent such as a long chain alkylamine hydrohalide. In such manner, the surface active agent permeates and thereby becomes affixed to the resin surface. In addition, the hydrocarbon portion of the surface-active agent is thought to become bound to the surface of the plastics. Subsequent digestion of the plastics articles with an aqueous solution of heparin provides articles of enhanced antithrombogenicity.

A further improvement is described in US Patent No 3,810,781 to Eriksson, wherein heparinized plastics surfaces are stabilized by cross-linking the bonded heparin molecules with dialdehydes. Some improvement in stability results, but not all of the bonded heparin is impervious to desorption by washing.

The binding of substantially greater quantities of heparin to the polymer surfaces is described in US Patent No 4,349,467 to Dudley, wherein the step of heparinization is carried out with aqueous solutions of heparin of 5% or greater concentration. Using this procedure, up to 18 microgram/cm of heparin is bound to a polyurethane surface through the surface active agent.

Many of the drawbacks and disadvantages of earlier methods for rendering polymeric surfaces nonthrombogenic are eliminated by the teaching of the above-named patents.

There remains, however, a need for methods to adhere higher quantities of heparin, impervious to desorption, to polymeric surfaces for use in articles which will be in contact with blood for prolonged periods of time.

These needs are met by the method of the current invention incorporating a plasma treatment of the resin surface before the heparinization step.

The process of formation of a plasma by electromagnetic activation of a gas by either a glow discharge or a corona discharge, and the use of such plasma for activating polymeric surfaces is known to accomplish various purposes. For example, US Patent No 3,663,265 to Lee teaches deposition of vaporized polymeric material onto substrate surfaces by treatment of the polymeric material with a plasma from an inert gas whereby the polymer is vaporized and contacted with the substrate to form the coating. In US Patent No 4,091,166 to Kubacki, a plastics surface is treated with a plasma of boron trifluoride, optionally admixed with an organic monomer to deposit a boron trifluoride containing coating. US Patent No 3,415,683 to Coffman discloses formation of deposits from organic materials on substrates such as bar metals in a corona reaction. US Patent No 3,518,108 to Heiss teaches formation of polymer coatings from aromatics, aliphatics and silicones in a plasma of inert gas or hydrogen.

US Patent No 3,776,762 to Bernath teaches glow discharge deposition of fluorocarbon polymeric coatings onto metallic or non-metallic surfaces by passing DC current through a low pressure atmosphere of the monomer. US Patent No 4,326,532 to Hammar discloses the use of a plasma for priming the surface of a polymeric resin for deposition of a coating of chitosan receptive to subsequent heparin binding.

The invention comprises a method to improve adherence of heparin to a polymeric surface. The polymeric article heparinized according to the present invention typically has improved compatibility with blood due to the increased level of heparin that can be adhered to the surface. The improvement results from a plasma treatment of the plastics surface before the heparinization step.

The plasma can be generated by ionization of a gas at low pressure by a radio frequency discharge. The frequency and power used to generate the plasma and the time of plasma treatment are variable over wide ranges. The plasma-treated polymeric surface thus obtained can then be subjected to heparinization according to known methods, whereby up to 10-fold more heparin is bound to the polymeric surface than occurs with untreated polymers. In addition, the heparin is usually bound more firmly, affording highly durable heparin surfaces which are resistant to removal of the heparin by such operations as washing or rubbing. Another advantage of the present invention is that normally no effect on the mechanical or penetration characteristics of prosthetic devices such as catheters is seen. Furthermore, in contrast to prior art methodology, the process of the present invention is relatively inexpensive and clean.

The polymeric resin materials which serve as the substrate to be treated by the method of this invention may be a polymeric resin, natural or synthetic, conventionally used to fabricate articles commonly used in contact with blood. For example, catheters, artificial blood vessels, valves and like prosthetics are frequently fabricated from a polyethylene, polyacrylic, polypropylene, polyvinyl chloride, polyamide, polyurethane, polyvinylpyrrolidone, polyvinyl alcohol, cellulose acetate, polystyrene, polytetrafluorethylene, polyester such as polyethylene terephthalate, silicone rubber, natural rubber, polycarbonate and like polymeric resins and hydrogels, thereof. The resin substrate may be rigid or flexible in character, cellular or non-cellular, porous or non-porous. Also within the scope of the invention is the treatment of a coating of such a polymer resin on a metal or ceramic material.

The polymeric resin substrate may be first formed into any desired shape, size or configuration. Representative forms include valves, pins, containers, sleeves, connectors, medical-surgical tubing, prosthetic devices and the like of any size. Alternatively, the polymeric resin may be first treated by the method of this invention and subsequently fabricated into the desired form.

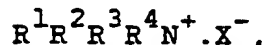
In accordance with the method of this invention, the polymeric resin substrate is subjected to a plasma treatment before heparinization. This plasma treatment may be carried out in a plasma generator, as, for example, those described in US Patent No 3,847,652. The plasma may be generated from a variety of gases or mixtures thereof. Gases frequently used include hydrogen, helium, ammonia, nitrogen, oxygen, neon, argon, krypton and xenon. Gas pressures are advantageously maintained at 5 mm of Hg or below, preferably from about 0.1 to about 1.0 mm of Hg, more preferably 10 to 300 micron of Hg, in order to benefit from reduced voltage requirements.

A wide range of power settings, radio frequencies and durations of exposure of the polymeric surface to the plasma may be used. Ranges for these three parameters which provide advantageous results are DC or AC power levels from 10 up to about 200 watts, from about 1 to about 50 megahertz and from about 0.1 to about 30 minutes, respectively. Preferred ranges are 10-50 watts, 10-20 megahertz and 2-10 minutes respectively.

The plasma-treated polymer can then be treated with a surface active agent before heparinization. For example, to the plasma surface thus activated by the plasma treatment there can be affixed by absorption on



the surface a quaternary ammonium salt such as one of the general formula (I),



wherein  $R^1$  is alkyl of 12-18 carbon atoms,  $R^2$ ,  $R^3$  and  $R^4$  are each independently selected from hydrogen atoms and lower alkyl groups of 1 to 6 carbon atoms, and X is for instance a negative monovalent ion, such as halogen. In preferred embodiments of this invention, the group  $R^2$  is lower alkyl and  $R^3$  and  $R^4$  are hydrogen. In a particularly preferred embodiment,  $R^2$  is methyl,  $R^3$  and  $R^4$  are hydrogen and X is chlorine.

Specific examples for the surface active agent include dodecylmethyammonium chloride, tetradecylmethyammonium chloride, hexadecylmethyammonium chloride, octadecylmethyammonium chloride or dodecylhexyammonium chloride, especially dodecylmethyammonium chloride.

The compound of Formula (I) is affixed to the polymeric resin substrate by permeating the compound throughout the molecular structure of the resin substrate, that is by chemisorption. While not willing to be bound by any theory, we believe that the  $C_{12}$  to  $C_{18}$  alkyl chain portion of the compound of formula (I) may also bind to the polymeric resin substrate which has been chemically altered by the plasma treatment. The compound (I) may be chemisorbed into the polymeric resin substrate by steeping the substrate in a dispersion of the compound

(I). In this steeping operation, the concentration of compound (I) is not critical, but is advantageously maintained within the range of 0.01% to 2.0% by weight. Steeping may be suitably carried out for 1 to 24 hours and at ambient or at elevated temperatures up to or slightly above the softening point temperature for the resin substrate, the softening point temperature being the temperature at which the surface of the resin substrate becomes pliable due to the additional mobility of the substrate molecules. Following fixation of the quarternary ammonium salt compound (I) to the surface of the polymeric resin substrate, excess compound (I) may be removed by washing with distilled water or saline solutions.

After the optional fixation, the polymeric resin substrate is preferably heparinized by immersion in an aqueous solution of sodium heparin. The temperature at which immersion is advantageously carried out is within the range of from about room temperature to about 80°C, but less than the softening point temperature for the resin substrate. The length of immersion is dependent on the temperature used, and might be from 1 to 24 hours, but is generally long enough to permit the substrate to pick up at least about 0.1 International Unit of heparin per square centimeter of substrate surface. At a temperature of about 70°C, for example, this is usually accomplished in about 1 hour, using a

heparin solution with a concentration of from about 1% to about 15%, preferably from about 8 to about 10%, of sodium heparin (all percentages herein are by weight unless otherwise indicated.)

During the preferred heparinization, the negative ion of the sodium heparin complexes with the positive ion of the compound I according to the scheme:



wherein A represents the active heparin moiety, that is, the negative ion of a salt of heparin, and  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$  and X are as previously defined.

Following the heparinization step, the polymeric resin can be removed from the heparin and rinsed thoroughly with distilled water.

The heparinized surface of the polymeric resin can be stabilized toward desorption in the presence of blood by treatment with a dialdehyde to cross-link functional groups of heparin. This cross-linking of functional groups in different heparin units is suitably accomplished when the heparinized surface is digested with an aqueous solution of a dialdehyde over a

concentration range of 0.1% to 5.0%. The aldehyde is preferably one with 2 to 6 carbon atoms, for instance glutaraldehyde. It is most advantageous to maintain contact between the heparinized surface and the dialdehyde solution for a time period of about 1 to about 6 hours at a temperature of ambient to about 80°C. The heparinized surface thus stabilized is preferably removed from the bath, washed thoroughly with distilled water and dried before being brought into contact with blood.

In a preferred embodiment of the present invention, a polytetrafluoroethylene or polyethylene substrate is maintained in an oxygen plasma generated at about 13.56 MHz and about 50 watts for about 10 minutes. The activated substrate is then steeped for about 16 hours at about 65°C in an aqueous approximately 15% solution of dodecylmethyl ammonium chloride. After washing with distilled water, the substrate with its affixed quarternary ammonium salt is treated at about 65°C for about 16 hours with an approximately 9% solution of sodium heparin. After washing with distilled water, the stabilization step is carried out with an approximately 1% aqueous solution of glutaraldehyde at about 60°C for about 2 hours.

The amount of heparin bound to the surface of the polymeric resin substrate can be determined by a standard procedure based on the quantitative removal of the dye Azure A by the reactive sites of the bound heparin. An example of a suitable methodology is as follows. Heparinized polymeric substrate of known surface area (between 2 and 35 cm<sup>2</sup>) is exposed to 5 ml of a 0.001% aqueous solution of Azure A for 45 minutes at 25°C. The quantity of dye removed from the solution is determined by spectrophotometric readings made at 630 nm, with a light path of 1 cm. The dye removed is converted to equivalent amounts of heparin by means of a standard curve prepared by reacting graded amounts of heparin (1-100 micrograms) with five ml of 0.001% Azure A in water, removing the insoluble heparin dye complex by extraction with 4 ml of cyclohexane, and quantifying the amounts of dye removed spectrophotometrically. The standard curve is then prepared by plotting amount of heparin added versus absorbance at 630 nm. The amount of heparin present on the tubing can then be determined by dividing the amount of heparin removed (derived from the standard curve) by the total surface area of the sample.

Polytetrafluoroethylene articles heparinized according to the method of the present invention were tested for blood compatibility by the procedure described in our US Patent 4,367,749, and found to be very satisfactory.

The following examples are provided to illustrate further the advantages of this invention, but the conditions and materials used and the amounts thereof are not limiting of the scope of the invention.

#### EXAMPLE 1

One hundred 1.25 inch (say 3.2 cm) radiopaque polytetrafluoroethylene catheters each weighing 20 g were placed in a plasma generator. The system was evacuated for 6 minutes to a pressure of 120 micron of Hg, then an oxygen bleed was started and maintained for 1 minute at a pressure of 180 micron of Hg. A plasma was initiated and maintained at 13.56 MHz and 50 watts power for 10 minutes. The chamber was air quenched, opened, and the catheters heparinized according to the following sequential three-step procedure.

1. Steeping in a 15% aqueous solution of dodecamethylmethyl ammonium chloride, pH 7.5, 16 hours, 65°C, followed by thorough rinsing in distilled water and drying with nitrogen.

2. Digestion in a 9% aqueous solution of sodium heparin for 16 hours at 65°C, followed by thorough rinsing with distilled water and drying with nitrogen.

3. Submersion in a 1% aqueous solution of glutaraldehyde for 2 hours at 60°C, followed by thorough rinsing with distilled water, 5% aqueous Triton x 405, and distilled water. The catheters were dried with nitrogen and stored in a vacuum oven at 25°C.

#### EXAMPLE 2

A polystyrene microtiter was placed in a plasma generator and the system evacuated to 20 micron of Hg. Ammonia was bled in at such a rate that a pressure of 200 micron of Hg was established and maintained. After a 5 minute flush at this pressure, a plasma was initiated at a frequency of 20 MHz and 40 watts power. The plasma was maintained for 5 minutes. The radio frequency was turned off and, after an additional 5 minutes flow of ammonia, the system was opened, the sample removed and heparinized as described in Example 1.

#### EXAMPLE 3

Twelve polytetrafluoroethylene catheters were placed in the plasma generator. The system was evacuated, and an oxygen bleed of 20 ml per minute giving a pressure of 180 micron of Hg was maintained for 2 minutes. A plasma was generated and maintained for 10 minutes at 13.56 MHz and 50 watts power. The system was quenched with air and the catheters removed and heparinized as in Example 1.

## EXAMPLE 4

Polytetrafluoroethylene tubing, 22 gauge, was cut into 3 foot (say 76cm) lengths and placed in a glow discharge chamber. The system was evacuated for 5 minutes and flushed with oxygen at 1 mm of Hg for 30 seconds. A flow of oxygen to produce a pressure of 180 micron of Hg was initiated, and plasma was generated for 10 minutes at 50 watts and 13.56 MHz. The system was quenched with air, opened, the substrate repositioned in the chamber to ensure total exposure to the plasma, and the plasma generated a second time in the same way. The tubing was removed from the chamber and heparinized as in Example 1.

## EXAMPLE 5

Into a plasma generator were placed the following:

- 8    20 g, 2.5 inch (say 6.4 cm) radiopaque  
polytetrafluoroethylene catheters (non  
siliconized)
- 4    16 g, clear polytetrafluoroethylene catheters
- 10   16 g polytetrafluoroethylene guidewires
- 10   pieces of polytetrafluoroethylene tubing



The chamber was evacuated and an oxygen bleed giving a pressure of 180 micron of Hg was maintained for 2 minutes. A plasma was then initiated using 50 watts of power and a frequency of 13.56 MHz maintained for 10 minutes. These materials were heparinized according to the procedure of Example 1. When these materials were exposed to a 0.001% aqueous solution of Azure A, heavy staining occurred, indicating high surface concentrations of heparin.

#### EXAMPLE 6

Into a plasma generator were placed a group of 2, 2.5 and 4 inch (say 5.1, 6.4 and 10.2 cm) radiopaque polytetrafluoroethylene catheters and 5 polytetrafluoroethylene coated guide wires. These were subjected to an oxygen plasma according to the procedure described in Example 5, and the plasma treated materials were heparinized according to the procedure of Example 1. The heparinized surfaces were subjected to an amount of rubbing equivalent to that which occurs in a typical human insertion. By comparison with samples which were not rubbed, it is seen, based on the Azure A staining procedure, that the heparinized surface retains most of the heparin after rubbing. This indicates a very durable heparin surface.

## EXAMPLE 7

This experiment was run in duplicate, giving the results shown in the following chart, runs 1 and 2.

Four 4-foot (say 102cm) samples each of radiopaque polytetrafluoroethylene (ptfe) tubing and clear polyethylene tubing were subjected to an oxygen plasma generated at 50 watts of power and a frequency of 13.56 MHz. Treatment durations of 1 and 10 minutes and oxygen pressures of 170 micron of Hg and 500 micron of Hg were used. The samples were heparinized according to the procedure of Example 1, except glutaraldehyde cross linking was not done. The tubing lumens were washed with hot 6 m sodium chloride solution to remove all heparin thereon, since it is desired to quantitate only the heparin on the outer surface of the tubings (which were exposed to plasma). The following chart summarizes the quantities of heparin bound to the surfaces of the tubing.

substrate	oxygen plasma treatment		bound heparin microg./cm <sup>2</sup>		
	time min.	pressure micron Hg	run 1	run 2	control*
polyethylene	1	170	5.70	6.77	7.37
polyethylene	10	170	5.74	6.82	
polyethylene	1	500	8.71	10.30	
polyethylene	10	500	7.94	9.39	
ptfe	1	170	1.24	1.50	0.22
ptfe	10	170	2.31	2.76	
ptfe	1	500	1.66	2.01	
ptfe	10	500	4.63	5.51	

\* heparin bound to substrate not subjected to oxygen plasma.

#### EXAMPLE 8

Polytetrafluoroethylene tubing of .02 mm inside diameter was placed in a plasma generator and the system was evacuated. Nitrogen was bled in at such a rate that a pressure of 180 micron of Hg was established and maintained. After a 2 minute flush at this pressure, a plasma was generated and maintained for 10 minutes at 40

watts of power and a frequency of 13.56 MHz. The radio frequency was turned off and, after an additional 10 minutes flow of nitrogen, the system was opened, the samples removed and heparinized as described in Example 1. The articles thus treated with plasma followed by heparinization were compared for blood compatibility with untreated controls and with controls heparinized without plasma treatment. The results of these comparative experiments are shown in Chart II.

Chart II

<u>untreated teflon controls</u>	<u>heparinized (no plasma pretreatment)</u>
9.4 minutes	18 minutes
13.2	22
13.8	26
15.2	29
15.7	59
Average = 13.5	Average = 30.8 minutes

heparinized (with plasma pretreatment)

47 minutes  
 92  
 120+ (truncated)  
 136  
 139

Average = 106.8 minutes

Thus an average 8 fold increase in blood compatibility over untreated controls and a 3-4 fold increase over heparinized articles not pretreated with plasma were achieved.

## CLAIMS

1. A method of reducing thrombogenicity by attaching heparin to the surface of a polymeric substrate, characterized by subjecting the substrate to a plasma treatment before attaching the heparin.
2. A method according to Claim 1 in which the polymeric substrate comprises silicone, polyethylene, polypropylene, polyurethane, polyacrylic, polyamide, polyester, polyvinyl pyrrolidone, polyvinyl alcohol, cellulose acetate, polyvinyl chloride or polytetrafluoroethylene, preferably polytetrafluoroethylene or polyethylene.
3. A method according to Claim 1 or 2 wherein the plasma is generated from a gas which comprises oxygen, ammonia, hydrogen, helium, neon, argon, krypton, xenon, nitrogen or mixtures thereof.
4. A method according to Claim 3 wherein the gas is maintained at a chamber pressure of from 10 to 300 micron of mercury during generation of the plasma.
5. A method according to any preceding Claim wherein the plasma is generated using a radio frequency discharge, preferably from 1 to 50 megahertz.

6. A method according to Claim 5 wherein the plasma is generated with a power of from 10 to 200 watts.
7. A method according to any preceding Claim wherein the polymeric substrate is subjected to the plasma for a period of from 0.1 to 30 minutes.
8. A method according to Claim 1 wherein a polytetrafluoroethylene substrate is subjected to an oxygen plasma generated by a discharge of about 50 watts at a radio frequency of about 13.56 megahertz and maintained for about 10 minutes with the oxygen at a pressure of about 180 micron of Hg.
9. A method for binding heparin to a polymeric substrate comprising the sequential steps of (1) subjecting the polymeric substrate to a plasma treatment, (2) steeping the plasma treated substrate in an aqueous dispersion of a cationic surface active agent, (3) immersing the substrate in an aqueous solution of sodium heparin, and (4) treating the substrate with an aqueous solution of a dialdehyde.

10. A method according to Claim 9 in which the cationic surface active agent is a compound of the formula  $R^1R^2R^3R^4N^+.X^-$ , wherein  $R^1$  is alkyl of 12-18 carbon atoms,  $R^2$ ,  $R^3$  and  $R^4$  are each independently selected from hydrogen and lower alkyl of 1 to 6 carbon atoms, and  $X^-$  is a negative monovalent ion.

11. A method according to Claim 10 wherein  $R^2$  is lower alkyl of 1 to 6 carbon atoms,  $R^3$  and  $R^4$  are each hydrogen and  $X^-$  is halide, preferably chloride.

12. A method according to Claim 11 wherein the surface active agent comprises dodecylmethyammonium chloride, tetradecylmethyammonium chloride, hexadecylmethyammonium chloride, octadecylmethyammonium chloride or dodecylhexyammonium chloride, preferably dodecylmethyammonium chloride.

13. A method according to any of Claims 9 to 12 wherein the aqueous concentration of the cationic surface active agent is from 0.01 to 2.0%.



14. A method according to any of Claims 9 to 13 wherein the steeping of the substrate in the aqueous dispersion of the surface active agent is carried out for 1 to 24 hours and/or the steeping is carried out at ambient temperature or at an elevated temperature up to or slightly above the softening temperature of the substrate.
15. A method according to any of Claims 9 to 14 wherein the substrate is immersed in aqueous solution of from 1 to 15 per cent of sodium heparin.
16. A method according to any of claims 9 to 15 wherein the immersion is carried out at a temperature from ambient temperature to 80°C and/or for 1 hour to 24 hours.
17. A method according to any of claims 9 to 16 wherein the dialdehyde contains from 2 to 6 carbon atoms and is preferably glutaraldehyde.
18. A method according to any of claims 9 to 17 wherein the dialdehyde is used in an aqueous solution of from 0.01 to 5% and/or the temperature of the dialdehyde aqueous solution is from ambient temperature to 80°C.

19. A method according to any of claims 9 to 18 wherein the substrate is contacted with the dialdehyde aqueous solution for from 1 to 6 hours.

20. A method for reducing thrombogenicity of a substrate, the method comprising the sequential steps of:  
(1) subjecting a polytetrafluoroethylene substrate to an oxygen plasma wherein the plasma is generated by a discharge of about 50 watts at a radio frequency of about 13.56 megahertz, the discharge being maintained for about 10 minutes with the oxygen at a pressure of about 180 micron of Hg;

(2) steeping the substrate in an aqueous solution of dodecylmethyl ammonium chloride of about 15% concentration for about 16 hours at about 65°C;

(3) washing the substrate thoroughly in distilled water;

(4) immersing the substrate in an aqueous solution of sodium heparin of about 9% concentration for about 16 hours at about 65°C;

(5) washing the substrate thoroughly in distilled water;

(6) digesting the substrate in an aqueous solution of glutaraldehyde of about 1% concentration for about 2 hours at about 60°C;

(7) washing the substrate in distilled water.